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14. ABSTRACT:

Decompensation in hemorrhagic shock is the critical stage after which resuscitative efforts may prove futile. We hypothesize that decompensation results from K+-mediated vasodilation and/or loss of cardiac contractility. Anesthetized rats were bled to a constant mean arterial pressure of 40 mmHg and subsequent resuscitated with normal saline at early (pre-decompensatory) and late (decompensatory) stages of shock. In the first set of experiments, microdialysis probes were implanted in skeletal muscle for continuous assessment of potassium and other metabolic markers. In a second set of experiments, animals underwent left heart catheterization and continuous measurement of cardiac contractility during hemorrhage and resuscitation. At the end of experiments, tissues were harvested for ex vivo Na+,K+-ATPase (NKA) activity. K+ concentrations in muscle interstitium were significantly higher in hemorrhaged animals than controls (2.34 times baseline vs. 1.24, $p < 0.05$), this difference was not reflected in blood values. NKA in early and late hemorrhagic shock was increased vs. controls in skeletal and cardiac muscle. Cardiac contractility fell with hemorrhage but was restored with resuscitation in both early and late shock. These results suggest that decompensation results from a loss of peripheral rather than cardiac responsiveness.

15. SUBJECT TERMS
Shock, Hemorrhage, Decompensation, Potassium, Electrolytes, Interstitium, Microdialysis

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INTRODUCTION

Decompensation is the critical "point of no return" in hemorrhagic shock. It is defined as sustained hypoperfusion leading to irreversible cardiovascular collapse (Pope *et al*., 1999), and is manifested clinically as the loss of ability to mount a hemodynamic response to aggressive fluid resuscitation. It is generally stated that once decompensation is reached, further resuscitative efforts are futile (Pope *et al*., 1999). Several chemical substances have been proposed as markers for tracking the evolution of hemorrhagic shock and impending collapse; these include pH, lactate, pyruvate, glucose, and base excess. An important military goal of metabolic monitoring would thus be identifying the most critical factors heralding decompensation in battlefield casualties. In addition to providing the ability to assess the stage or severity of hemorrhagic shock, such monitoring may establish prognosis and/or determine the adequacy of therapeutic interventions.

The relationship between serum concentrations—which are commonly measured experimentally and in controlled clinical settings—and the corresponding concentrations in the tissue interstitium has not been explored systematically. This has important consequences for metabolic monitoring because it may be faster and more practical on the battlefield to sample from a transcutaneous or intramuscular sensor than to attempt intravascular sampling. Additionally, the interstitial level may be more representative of the end-organ milieu—and thus a better indicator of underlying pathophysiology. Further information is thus needed about the correlation (or lack thereof) between interstitial and intravascular concentration with regards to monitoring for decompensation.

It is possible that these substances serve not just as indicators, but also as actual mediators of the pathogenesis of decompensation. Specifically, muscle interstitial potassium has been shown to rise out of proportion to intravascular levels during hemorrhage (Illner and Shires, 1980; McKinley *et al*., 1981). These changes are consistent with an effective loss of Na^+-K^+ ATPase (NKA) activity. Hyperkalemia may be a mechanism for vascular smooth muscle hyper-polarization and vasodilatation observed at the decompensatory stage (Stekiel *et al*., 1980; Lombard *et al*., 1986), which can be mediated by the inwardly rectifying potassium Kir channels or by NKA (Nelson and Quayle, 1996; Savage *et al*., 2003).

Our objectives were to revisit the issue of interstitial changes in potassium and other metabolic substances (glucose, lactate, pyruvate, and glycerol) in the pathogenesis of hemorrhagic shock and the response to crystalloid resuscitation. In addition, we examined the *ex vivo* tissue NKA activity in skeletal and cardiac muscle and other tissues, as well as the plasma levels of circulating oubain-like NKA inhibitor. Finally we monitored changes in cardiac contractility with hemorrhage and resuscitation and their relationship to NKA and NKA inhibitor levels. Our hypothesis was that interstitial hyperkalemia in skeletal muscle may herald the onset of decompensation

BODY

Details on the background and experimental method may be found in the research proposal (MRMC Log No. 01155005). The following tasks composed the Statement of Work for Year Three:

1. **Continued refinement of experimental protocols as necessary.**

We sucessfully integrated a resuscitation stage into the experimental protocol and incorporated the measurement of cardiac contractility during hemorrhage and resuscitation experiments.

2. Conduct experiments in 40 animals.

Experiments were done on 51 animals done between March 2004 and October 2005. The total number of animals used for the duration of the funding was 166. A breakdown of the animals is presented in the results section.

3. Present analysis of correlations of interstitial and intravascular concentrations to the response to resuscitation.

The most recent resuscitation experiments using microdialysis measurements of interstitial concentrations were recently completed. Analysis of these samples is underway.

4. Present recommendations on development of metabolic probes based on the measurements of interstitial chemistries

Based on the data analyzed to date, a metabolic probe which provided interstitial potassium levels might serve as a useful diagnostic adjunct on the battlefield. The utility of interstitial lactate and glucose measurements is pending further analysis of more recent data from hemorrhage and resuscitation experiments.

METHODS

Animals
Male Wistar rats (Charles River Laboratories, Wilmington, MA) were quarantined for ten days in a temperature- and light- controlled environment. Animals had *ad libitum* access to rodent chow (Nestlé Purina, St. Louis, MO) and water. After quarantine, animals were weighed for five to seven days prior to use in order to document continued weight gain (5 g/day). Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations related to animals, and experiments involving animals adheres to principles stated in the Guide to the Care and Use of Laboratory Animals, National Research Council.

All control animals received microdialysis probe placement. Hemorrhaged animals received either microdialysis probe placement or placement of a catheter for cardiac contractility.

Surgical Preparation: Microdialysis
On the day of experimentation, the rats were initially anesthetized with sodium pentobarbital (50 mg/kg) intra-peritoneally. Once a surgical plane of anesthesia had been attained, the rats were shaved and the skin cleaned. A tracheostomy was performed using PE 240 tubing (Clay Adams, Inc., Parsippany, NJ).

Under aseptic conditions, catheters (PE50, Clay-Adams) were placed in the following locations: left femoral artery, left carotid artery, left femoral vein, and caudal right quadrant of the peritoneum. The left femoral artery catheter was attached to a continuous blood pressure

monitor (BPA, Micromed, Louisville, KY). The left carotid artery catheter was attached to a computer controlled peristaltic pump (Model 720, Instech Laboratories, Inc., Plymouth Meeting, MA) that emptied into a heparinized reservoir placed on a balance (PB303S with RS232 port, Mettler, Inc., Toledo, OH). The left femoral vein catheter was used for venous access as needed.

The peritoneal catheter was used to provide a continuous infusion of sodium pentobarbital (10% in normal saline at 0.06 μl/min/g) administered to maintain the anesthetic plane. Additional pentobarbital was administered as a 0.05-0.1 ml intraperitoneal bolus as needed to maintain loss of digital and corneal reflexes.

A rectal thermistor temperature probe was inserted and the core temperature was maintained at 37 ^{o}C by a homeothermic blanket (Harvard Apparatus, South Natick, MA) and heating lamp. Each animal was anticoagulated with porcine heparin (0.5 IU/g i.v. to a maximum 175 IU) prior to the start of the stabilization period.

Microdialysis probes (CMA/20, CMA/Microdialysis, North Chelmsford, MA) were placed in a branch of the right femoral vein, in the quadriceps major of the right leg, and in the liver through an abdominal incision.

Surgical Preparation: Cardiac Contractility
The animals undergoing contractility measurements had similar preparation to those undergoing microdialysis with the following exceptions: PE50 catheters were placed in the left femoral artery, right femoral artery, and left femoral vein. The left femoral artery catheter was connected to the pump and the right femoral artery catheter was connected to the BPA, while the left femoral vein catheter was used for access. A pressure transducer (Model No. SPR-249, 3F, output 5 μV/V/mmHg, Millar Instruments, Inc., Houston, TX) was passed through the right carotid artery into the left ventricle for continuous measurement of left ventricular pressure. The signal was sent to a differentiator (Model 13-G4615-71, Gould Electronics, Cleveland, OH) and recorded on a chart record (Model 35-V8808-00, Gould Electronics).

Hemorrhage and Resuscitation Protocol
The WRAIR Hemorrhagic Shock Data Acquisition (HSDAQ) Program, an interactive program written in LabVIEW (National Instruments, Austin, TX) controlled the hemorrhage. This program monitored arterial blood pressure and the weight of the shed blood volume (SBV) removed from the animal. It controlled the peristaltic pump to maintain blood pressure at the desired level. Arterial pressures (systolic, diastolic, and mean), heart rate, and shed blood volume were monitored continuously and recorded every 5 seconds by the program. Controlled hemorrhage was performed following our established protocol. The HSDAQ program was started and the animal was monitored initially for a 20-minute stabilization period after completion of the surgery. After the control period, the program commenced hemorrhage by withdrawing blood from the carotid artery into the reservoir. Mean arterial pressure (MAP) was linearly dropped to 40 mm Hg over a 15 minute time period, then maintained at that value for the duration of the experiment by the additional withdrawal or return of shed blood to the animal (with program providing feedback control). In the initial (compensatory) phase of hemorrhage, blood had to be continuously withdrawn from the animal to maintain the desired MAP. After a period, however, the shed blood had to be returned to the animal from the

reservoir to maintain this pressure. The point where this transition occurs is the start of decompensation, and thereafter the phase of shock is designated by the amount of blood that has been returned to the animal, expressed as a percentage of the peak SBV (Connett *et al.*, 1986). Experiments continued until return of a given fraction of the peak SBV.

The resuscitation groups received resuscitation with normal saline immediately after attainment of the target SBV. The saline was given at a rate to attain a MAP of 80 mmHg over 15 min, then to maintain it for an additional 30 min, at which point the experiment was stopped.

Control animals underwent the same surgical preparation as the bled animals but the peristaltic pump was never activated. The duration of the control experiments were matched to that of the preceding hemorrhage experiment.

Blood gases
Arterial blood samples were collected from the left femoral line at $t = -17$ min from the start of the bleed (during the stabilization period), at t = 43 min, and subsequently at thirty-minute intervals. Microhematocrit was measured in a centrifuge (Model TRIAC, Clay Adams). Arterial blood gases and potassium were measured using an i-STAT portable clinical analyzer (i-STAT Corp., East Windsor, NJ).

Microdialysis Sampling
Microdialysis was performed continuously during hemorrhage by perfusing the implanted probes with normal saline containing 4.5 mM RbCl. The perfusate rate was 1 μl/min and it was collected in 15-μl fractions by a fractional collector (CMA/142, CMA Microdialysis). Potassium concentrations in the microdialysis samples were measured using the internal standard technique (1) to correct for lack of 100% recovery in the probes: the fractional loss of rubidium from the perfusate was assumed equal to the fractional uptake of potassium into the perfusate from the surrounding tissue. 10-μl aliquots of the collected fractions were diluted 1:1000 in 10 ml of 2% HNO_3. Potassium and rubidium concentrations were then measured using inductively-coupled plasma mass spectrometry (Elan 6000, Perkin-Elmer, Norwalk, CT). Concentrations of glucose, lactate, pyruvate, and glycerol were measured in the remainder of the microdialysis samples using a commercial analyzer (CMA 600). Internal standards were not used for these analytes, thus the concentrations obtained are underestimates.

Tissue NKA activity and Plasma NKA Inhibitor
At the end of each experiment, the animals were euthanized and the following tissues were harvested: left quadriceps major, left heart ventricle, renal medulla, diaphragm, right lobe of liver, and 0.3 ml of blood (subsequently fractionated into red blood cells and plasma). These were prepared and microsomal fractions isolated as previously described (Pamnani *et al*, 2000). Briefly, ATPase activity was measured by the amount of inorganic phosphate liberated from ATP during a 1 hr incubation at 37 °C. Total ATPase activity was measured using an assay medium of 120 mM NaCl, 10 mM KCl, 40 mM Tris-HCl (pH 7.5 at 37 oC), 2 mM Tris-ATP, 2.5 mM MgCl2, and 0.5 mM EGTA. Mg^{2+}-ATPase activity was measured using the same assay medium except 1 mM ouabain was substituted for KCl. NKA activity was defined as total ATPase activity minus Mg2+-ATPase activity. Activity was normalized to total microsomal

protein measured by the Lowry assay. NKA inhibitor activity in the plasma was measured as inhibition of a standardized kidney NKA assay.

Cardiac Contractility
Cardiac contractility was measured from the analog recording from the chart recorder as left ventricular $(dP/dt)_{max}$.

Statistical Analysis
Statistical significance between groups was determined using two-tailed Student's test for comparing means of independent measurements or by one-way ANOVA as appropriate. Significance was defined as a *p* value < 0.05.

RESULTS

Animals
A total of 166 rats were used during the three-year period of funding. The breakdown of the experimental groups is given in Table 1. Part of the original experimental plan was to examine the differences between animals at various points in the decompensatory stage (25% vs. 50% vs. 100% return of peak SBV). Due to the extreme lengths of the longer experiments, and the feeling that the incremental value of times past 25% return of peak SBV, we instead chose to focus only on one group of animals in decompensatory shock, those at 25% return. These animals are designated the Late Shock (LS) group. Control animals that were matched to this group are designated LS controls. Hemorrhaged animals that were match-bled to 50% of the peak shed blood volume of the LS group are designated the Early Shock (ES) group. LS and ES animals that were subsequently resuscitated are designated LS+R and ES+R, respectively.

The data analysis will focus on the LS controls, LS, ES, LS+R, and ES+R groups.

Baseline and Hemodynamic Data
Baseline and hemodynamic data for each group is given in Table 2. Baseline data (weight, starting blood pressures) were similar for all groups. There were some minor various in due to changes in technique from older experiments.

Microdialysis Results
Figures 1-8 summarize data from LS and LS controls which has been analyzed to date. The data from LS+RW animals is still forthcoming.

Figure 1 shows average hemodynamic data (mean arterial pressure and shed blood volume) for a the late hemorrhage experiments vs. controls. The plots demonstrate how the HSDAQ program reproducibly maintains the hemorrhaged animals at the desired target pressure.

Figure 2 shows the average pH and change in base excess for the animals, providing an illustration of the severity of the shock administered by the hemorrhage

Shown in Figures 3-8 are arterial and microdialysis results for K^+ and various other metabolic parameters. To emphasize the changes with time, most values are shown as ratios to baseline.

Vertical lines represent the average time to peak shed blood volume.Potassium (Figure 3): Interstitial $[K^+]$ was higher in muscle at peak SBV than controls (ratio = 2.33 vs. 1.24 times baseline, $p < 0.05$); this was not reflected in vein or liver. These results are consistent with previous studies (Illner and Shires, 1980; McKinley et al., 1981). After peak SBV, average muscle $[K^+]$ declines vs. time due to drop-out of animals reaching experimental end. When vein and muscle levels in hemorrhaged rats are plotted as a scattergram vs. stage of shock (% of peak SBV bled, third panel of Figure 3), the slope of the linear correlation to the muscle data is larger (1.87 vs. 1.12).

Glucose (Figure 4): Arterial glucose ratios were higher (2.68 vs. 1.06, $p < 0.05$) at peak SBV than controls, then decreased. Similar trends were seen in venous and muscle samples, but did not reach significance. In liver samples no trend versus time for either groups was appreciated.

Lactate (Figure 5): Values in all tissues were higher in hemorrhaged animals, but only reached significance in venous samples at $t = 28$ (ratio = 3.05 vs. 1.42) and 43 (6.91 vs. 1.67) minutes. In muscle similar differences were seen (ratios = 2.8 vs. 0.8 @ 28 min and 4.9 vs. 0.8 @ 43 min) but hemorrhage vs. control differences were not significant due to small $n = 4$ from analytical problems. In liver the difference at t = 28 min just fell short of statistical significance (2.74 vs. 1.32, $p = 0.068$). The smaller relative increase in muscle and liver is consistent with previous results and has been used to argue that these tissues are lactate consumers in hemorrhage (Okuda et al., 1992).

Pyruvate (Figure 6): Venous samples tended to increase with time in both hemorrhage and control groups without statistically significant difference. Samples from muscle and liver in both groups showed no clear change with time.

L/P ratio (Figure 7): Trended upward with time in control liver samples and all hemorrhaged samples without significant statistical difference.

Glycerol (Figure 8): Levels in all tissues tended to increase in both hemorrhage and control animals, without significant difference between groups.

NKA and NKA Inhibitor Results
Fig. 9A: The computer program kept the blood pressure profiles for each of the four hemorrhage groups matched during the bleed phase. Error bars represent ± 1 standard error of the mean.

Fig. 9B: The shed blood volume curves during the bleed phase were comparable for the two early shock groups and the two late shock groups.

Fig. 9C: Results for the resuscitation phase are shown as scatter plots because the time at which resuscitation began was different for each animal. All ES+R animals reached the target MAP of 80 mmHg, several auto-resuscitated before the 15-minute goal. Only 2 of the 14 LS+R animals reached the target MAP. Five LS+R animals went into accelerated decompensation with the start of resuscitation and the experiments were terminated early.

Fig. 9D: As expected, significantly less fluid was required to resuscitate the ES+R rats vs. LS+R.

Skeletal Muscle (Fig. 10): In both ES and LS, skeletal muscle NKA activity increases showed significant increase in hemorrhage, then a significant decline with resuscitation.

Left Ventricle (Fig. 11): Cardiac muscle was unique in that it showed two different patterns in ES vs. LS. All groups showed increases in NKA activity from controls.   In LS, however, the activity was significantly diminished from ES. The response to resuscitation was also different in the two phases of shock. For ES, there was a non-significant trend toward NKA activity falling in ES+R, while in LS, there was a significant increase with LS+R.

Kidney (Fig. 12) : All groups showed increases in NKA activity with no clear trend among them.

Diaphragm (Fig. 13): NKA activity was significant from control only in LS.

Liver (Fig. 14) : No differences in NKA activity from control were seen in any of the groups.

Red blood cells (Fig. 15): NKA activity was decreases in both ES and LS, and tended to fall in both resuscitation groups. The change with resuscitation was only significant for ES+R, however.

Plasma NKA inhibitor (Fig. 16): inhibitor levels fell significantly from controls in all groups, and there was no apparent change with resuscitation.

Cardiac Contractility
7/8 of ES+R vs. 2/8 of LS+R rats were successfully resuscitated to target (p=0.04). (dP/dt)$_{max}$ fell equally in both groups with hemorrhage but returned to baseline with resuscitation (Table 3). A decrease in NKA-I in both ES and LS correlated with an increase in tissue NKA; these were unchanged with R.

KEY RESEARCH ACCOMPLISHMENTS

- Interstitial hyperkalemia appears to correlate with the onset of hemodynamic decompensation in hemorrhagic shock.
- *Ex vivo* ATPase activity is increased from controls in kidney, heart, skeletal muscle, diaphragm, and red blood cells in both late hemorrhagic shock (25-50% return of shed blood volume). In skeletal muscle and heart, it is also elevated in early hemorrhagic shock, suggesting the possibility that a fall from peak activity may correlate with the onset of decompensation.
- Plasma NKA inhibitor activity decreases in response to hemorrhage but appears unaffected by resuscitation.

- Resuscitation has varying effects in different tissues: it lowers NKA activity in skeletal muscle in both early and late shock, but increases NKA activity in cardiac muscle in late shock.
- The ability to respond to respond to resuscitative fluid is preserved in early hemorrhage but lost in the decompensatory (late) stage. This is not explained by changes in cardiac contractility.

REPORTABLE OUTCOMES

Articles

Oliver, J. D. III, J. L. Atkins, J. F. Schooley, E. R. Morris, L. Wang, T. B. Bentley, and M. B. Pamnani. Changes in interstitial metabolic parameters during hemorrhagic shock. In *Combat Casualty Care in Ground Based Tactical Situations: Trauma Technology and Emergency Medical Procedures*, RTO-MP-HFM-109. NATO Research and Technology Organization, 2004.

Published Abstracts

Oliver, III, J. D., J. L. Atkins, J. L. Schooley, E. R. Morris, L. Ma, T. B. Bentley, and M. B. Pamnani. Interstitial concentrations during hemorrhagic shock. *Shock*, **19 Suppl**:53, 2003.

Oliver III, J. D., J. L. Atkins, J. F. Schooley, L. Ma, T. B. Bentley, and M. B. Pamnani. Hemorrhagic Shock in Rats Increases Microsomal Sodium-Potassium ATPase (NKA) Activity *Ex Vivo. Shock*, **21 Suppl**:43, 2004.

Oliver III, J.D., J. F. Schooley, L. Wang, T. B. Bentley, J. L. Atkins, and M. B. Pamnani. Changes in *ex vivo* Na^+,-K^+-ATPase (NKA) in hemorrhage (H) and resuscitation (R). Shock, 2005.

Presentations

Date	Title	Meeting
24 Apr 02	Microdialysis (μD) Measurement Of Interstitial Potassium Concentrations During Hemorrhagic Shock	Experimental Biology New Orleans, LA
11 Sep 02	Measurement Of Interstitial And Intrahepatic Electrolytes During Hemorrhagic Shock Using Microdialysis And Inductively-Coupled Plasma Mass Spectometry (ICP-MS)	ATACCC St. Pete Beach, FL
15 Apr 03	Microdialysis (μD) Measurement Of Interstitial Markers of Hemorrhagic Shock	Experimental Biology San Diego, CA
09 Jun 03	Interstitial Concentrations During Hemorrhagic Shock	Shock Society Phoenix, AZ

CONCLUSIONS

[K+] in skeletal muscle during hemorrhage appears to correlate with the onset of decompensation, while intravascular [K+] does not. Changes in blood glucose also correlate with peak SBV. Muscle and liver glucose may be similarly correlated, although the magnitude of the change appears to be less. The rise in venous lactate levels from microdialysis also corresponded with peak SBV. Changes in tissue lactate had similar trends but did not reach statistical significance due to small numbers. Interstitial measures of potassium, lactate, and/or glucose may prove to be of diagnostic and prognostic significance in hemorrhagic shock. Interstitial hyperkalemia may be a physiological mechanism for decompensation..

Hemorrhagic shock increases the *ex vivo* NKA activity in most tissues. The rise in extracellular potassium seen in hemorrhage thus does not appear to be due to a reduction in the intrinsic pump capacity. Possible explanations include the presence of an *in vivo* NKA inhibitor, an uncoupling of NKA activity from potassium transport, or through effects of other potassium pathways. The mechanism for increases in NKA activity *in vivo* may be due to stimulation from catecholamines, high extracellular potassium, or intravascular volume depletion.

Hemorrhagic shock in most tissues results in an increase in *ex vivo* NKA activity and corresponding decrease in circulating NKA inhibitor. This suggests that the extracellular hyperkalemia is not due a reduction in the intrinsic pump capacity. Hyperkalemia must thus result from either an uncoupling of the NKA pump or from another mechanism. The decline in inhibitor concentrations during hemorrhage is consistent with the previously described phenomenon of elevated NKA inhibitor levels in states of hypervolemia.

The relative changes in ES vs. LS vary by tissue. Resuscitation tends to decrease tissue NKA with the notable exception of cardiac tissue in LS+R. NKA inhibitor levels, however, do not appear to be affected by resuscitation subsequent to hemorrhage. Failure reach target MAP with resuscitation after prolonged hemorrhage is not explainable by changes in $(dP/dt)_{max}$, NKA, or NKA-I

Of overriding interest is the difference in resuscitation success for animals in ES vs. LS groups. Our data suggests no difference in cardiac contractility, which implies decompensation after hemorrhage is due to peripheral mechanisms. Further studies investigating potassium's role, including therapies directed at inhibiting Kir channels or NKA, should be performed.

The factors that distinguish those LS animals which tolerate resuscitation better than others should also be an area of future investigation.
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APPENDICES

GROUP	SUBGROUP	Description	*n*
Control Total			**50**
(subtotals)	C100%SBVR	controls for H100%SBVR	13
	C50%SBVR	controls for H50%SBVR	9
	LS controls	controls for LS	28
Hemorrhage Total			**85**
(subtotals)	H100%SBVR	hemorrhage to 100% of peak SBV return	32
	H50%SBVR	hemorrhage to 50% of peak SBV return	11
	LS	"Late Shock" = hemorrhage to 25% of peak SBV return	27
	ES	"Early Shock" = hemorrhage to 50% of predicted peak SBV	15
Resuscitation Total			**31**
(subtotals)	LS+R microdialysis	LS + normal saline resuscitation with microdialysis studies	6
	LS+R contractility	LS + normal saline resuscitation with contractility studies	9
	ES+R microdialysis	ES + normal saline resuscitation with microdialysis studies	6
	ES+R contractility	ES + normal saline resuscitation with contractility studies	10

Table 1. Breakdown of experimental groups.

Group	n	Weight (g)	MAP_0 (mmHg)	HR_0 (mmHg)	SBP_0 (mmHg)	DBP_0 (mmHg)	pSBV (ml)	t-pSBV (min)	tRESUS (min)	VRESUS (ml)	TSURV (min)
H100%SBVR	32	366±8	87±4	397±10	113±5	74±4	5.5±0.5	39.8±4.4			112.5±13.3
C100%SBVR	13	367±14	97±5	379±13	123±7	83±4					
H50%SBVR	11	357±5	95±4	369±13	125±4	82±4	6.5±0.5	84.5±7.6			176.1±14.7
C50%SBVR	9	372±9	104±6	404±7	134±6	90±6					
LS	27	393±13	98±4	379±6	127±4	85±3	6.1±0.4	60.8±4.5			99.9±10.2
LS controls	28	393±12	90±4	370±10	119±5	77±4					
LS+R	15	380±10	93±3	372±8	122±4	79±3	8.2±0.3	88.3±2.9	154.3±9.8	28.5±2.9	188.2±12.1
ES	15	398±15	97±4	358±7	126±4	84±3	3.5±0.3	27.9±3.9			29.9±3.8
ES+R	16	369±7	102±3	374±6	133±4	88±3	4.2±0.2	32.8±3.8	32.6±3.8	6.0±1.8	80.5±3.9

Table 2. Baseline hemodynamic data for early hemorrhage groups: MAP_0 = mean arterial blood pressure at end of 20-minute control phase prior to bleed, HR_0 = heart rate at end of control phase, SBP_0 = systolic BP at end of control phase, DBP_0 = diastolic BP at end of control phase, pSBV = peak shed blood volume, t-pSBV = time to peak shed blood volume from start of bleed, tRESUS = time resuscitation started, VRESUS = total volume of normal saline given for resuscitation, TSURV = survival time
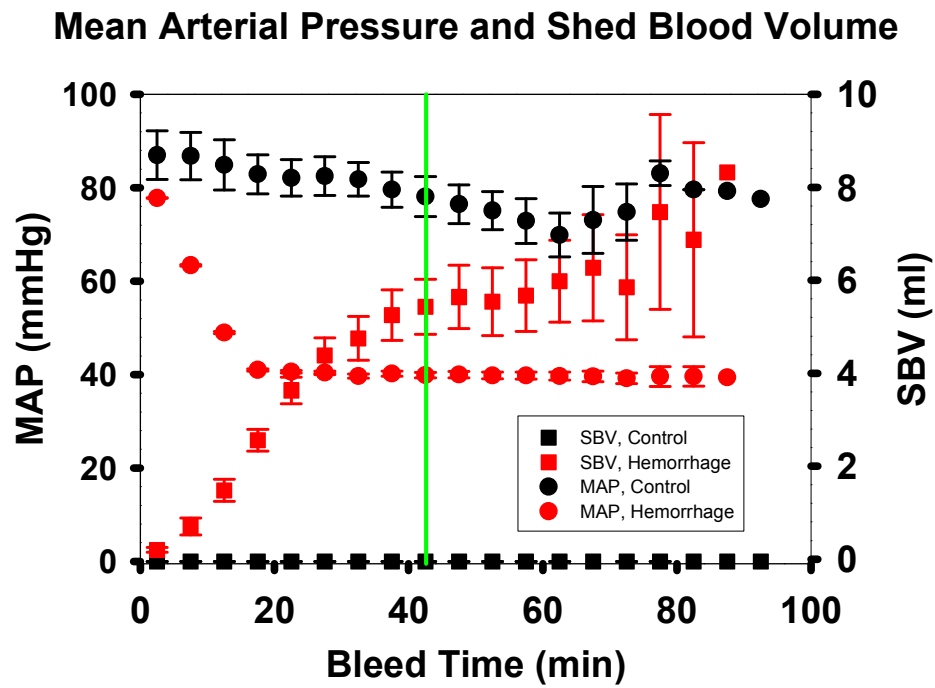
Figure 1. Average mean arterial pressure and shed blood volume for late hemorrhage (25% return of shed blood volume) animals. Vertical line represent average time to peak shed blood volume. Error bars represent ± one S.E.M.
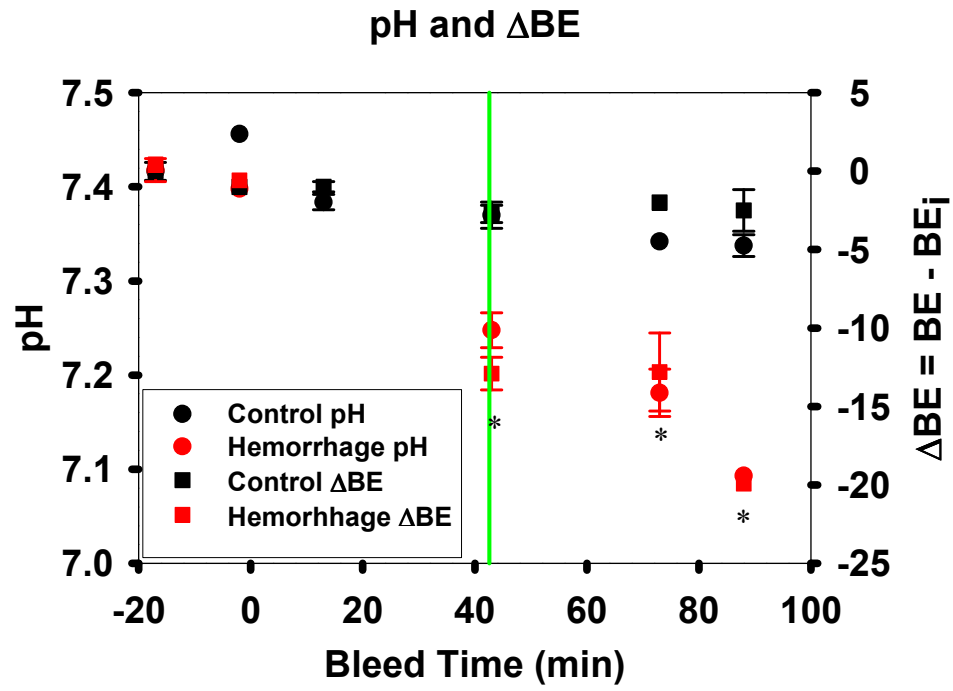
Figure 2. pH and change in base excess for hemorrhage vs. control animals. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.
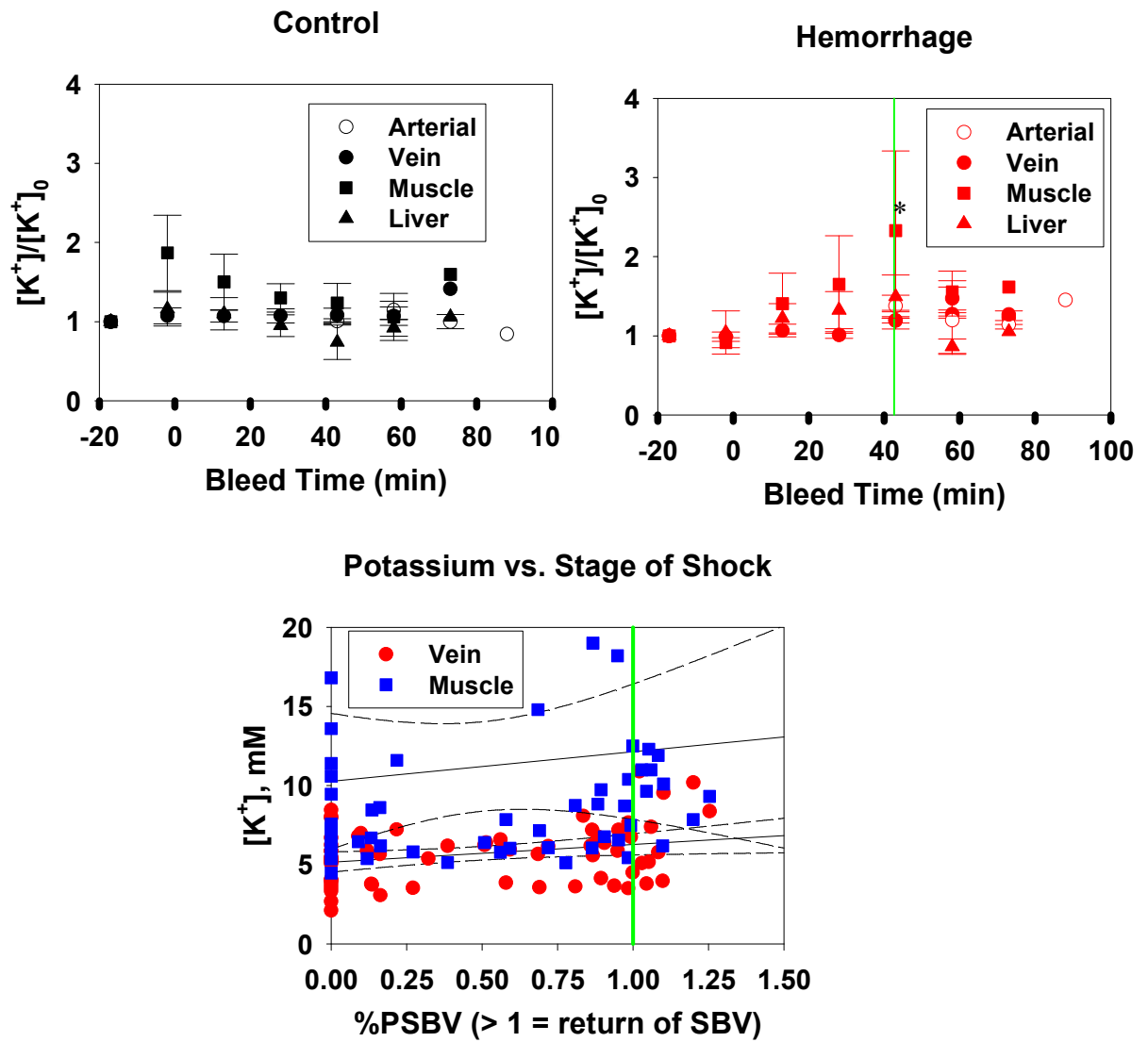
Figure 3. (Top panels) Potassium concentrations relative to baseline levels in arterial (open circles) and tissue microdialysis (closed symbols) samples (Bottom panel) Scattergram of potassium concentration vs. stage of shock (percentage bled of peak shed blood volume). Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.

Arterial Glucose

Control
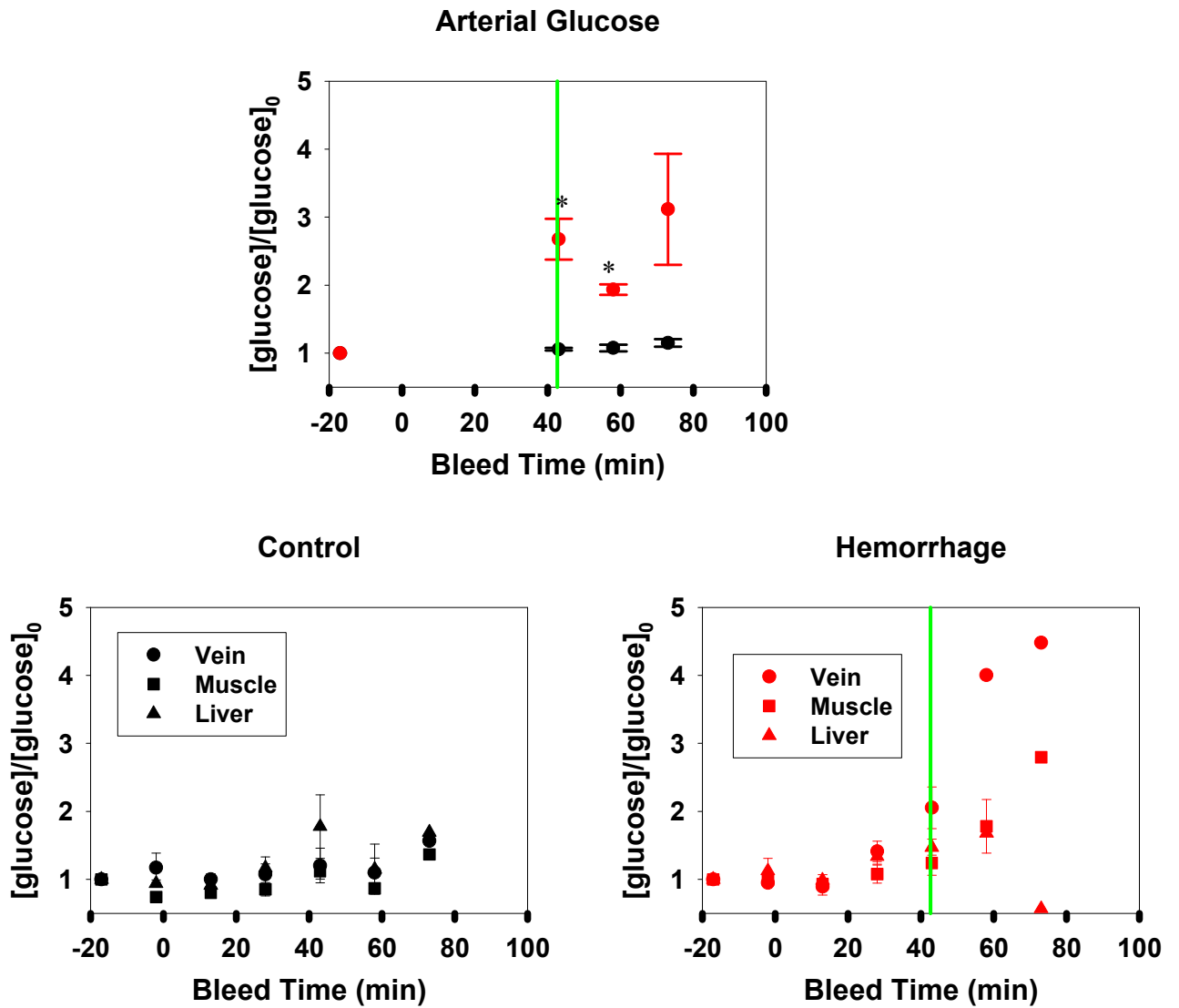
Hemorrhage

Figure 4. (Top panel) Relative arterial glucose concentrations for hemorrhage vs. controls. (Bottom panels) Relative glucose levels from microdialysis samples. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.
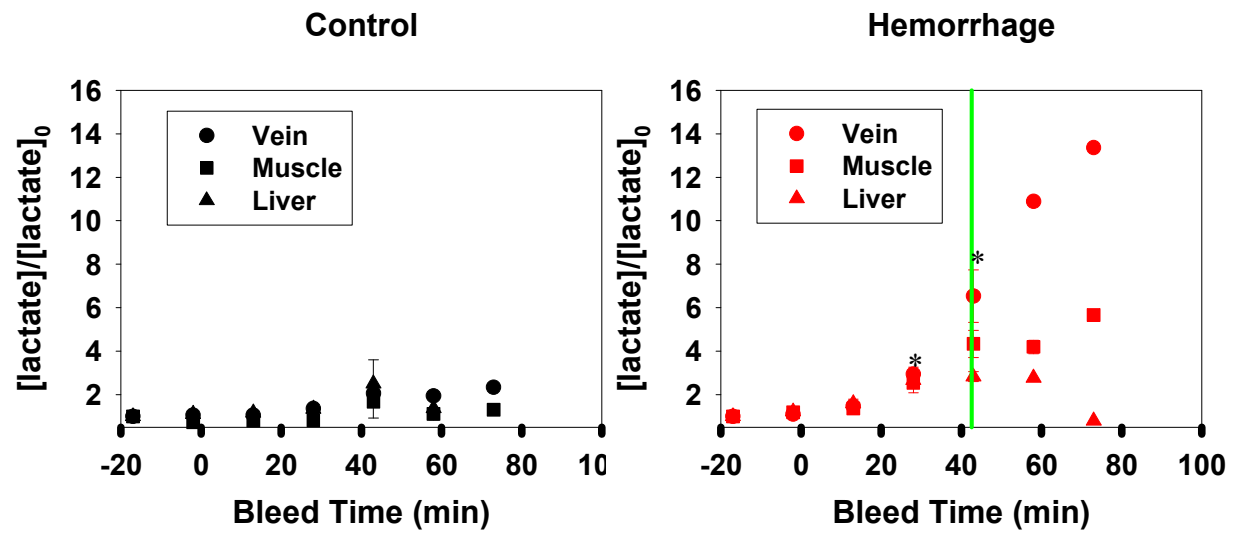
Figure 5. Relative lactate levels from microdialysis samples. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.
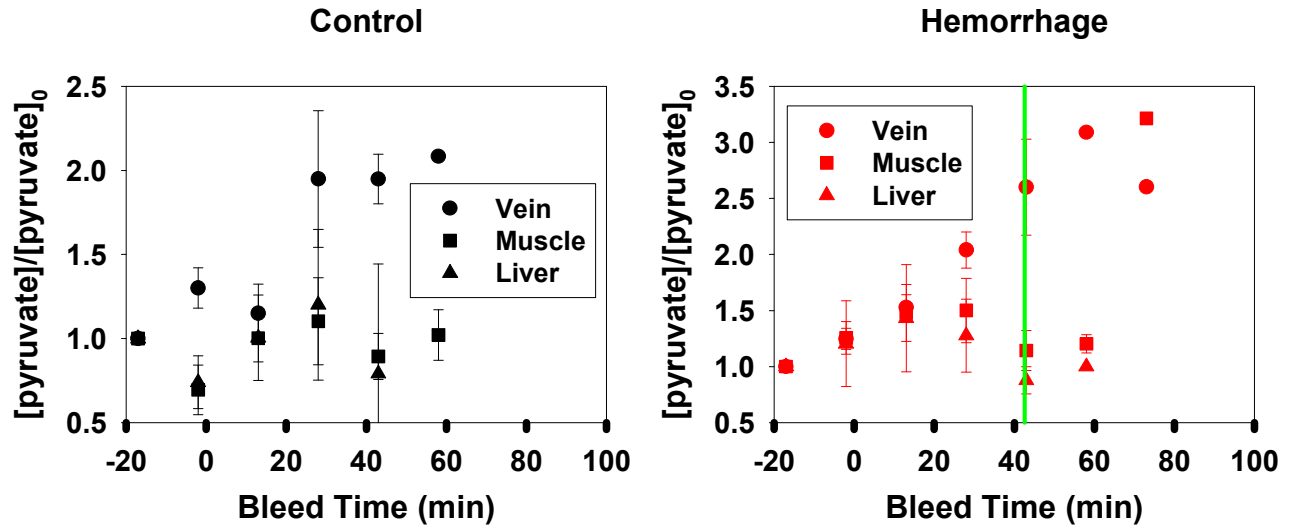
Figure 6. Relative pyruvate concentrations from microdialysis samples. Error bars represent ± one S.E.M. *p < 0.05 for hemorrhage versus control.
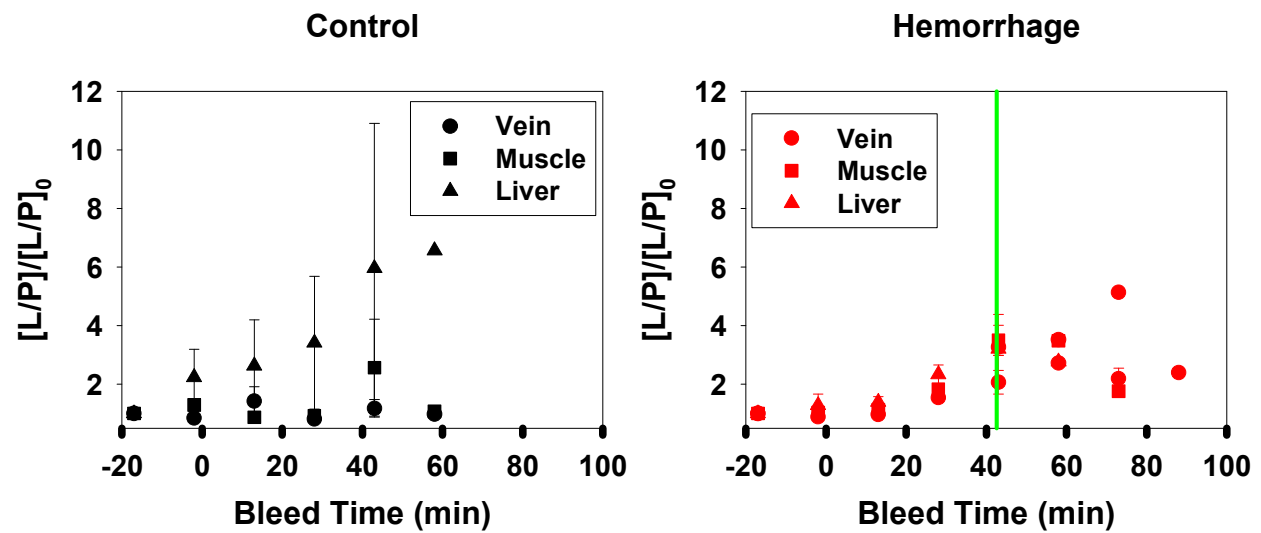
Figure 7. Relative lactate/pyruvate ratios from microdialysis samples. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.
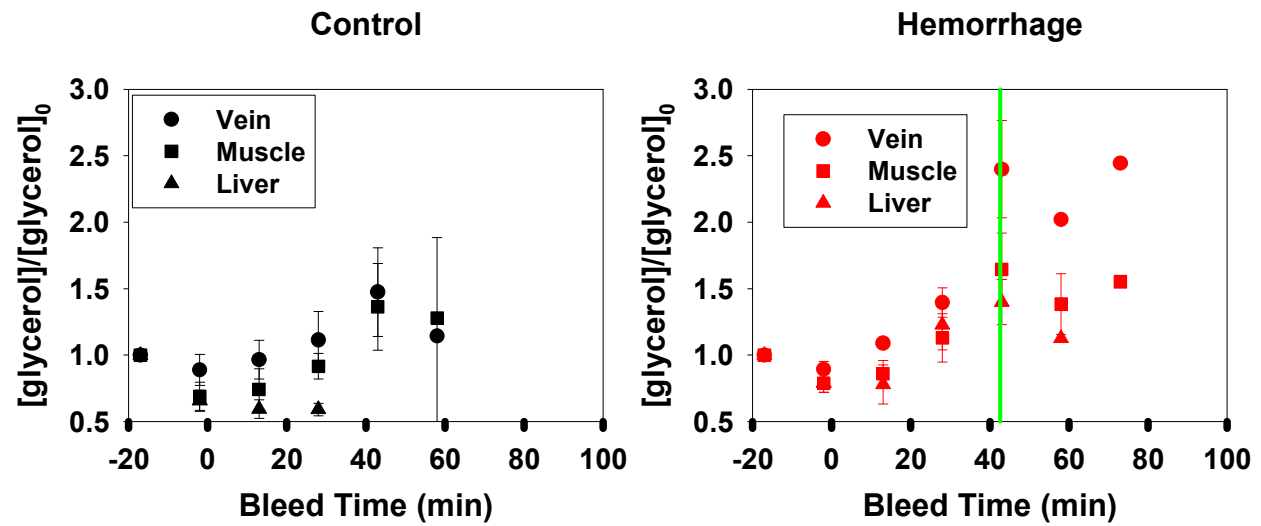
Figure 8. Relative glycerol concentrations from microdialysis samples. Error bars represent ± one S.E.M. *$p < 0.05$ for hemorrhage versus control.

A: Mean Arterial Pressure during Bleed Phase

B: Shed Blood Volume during Bleed Phase
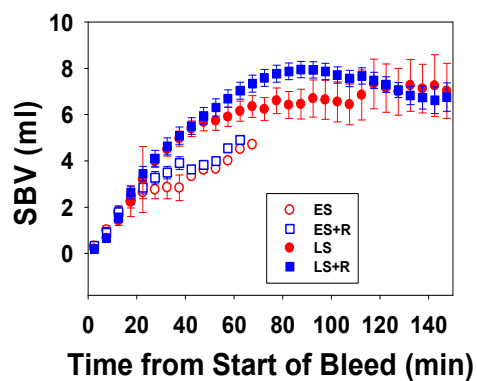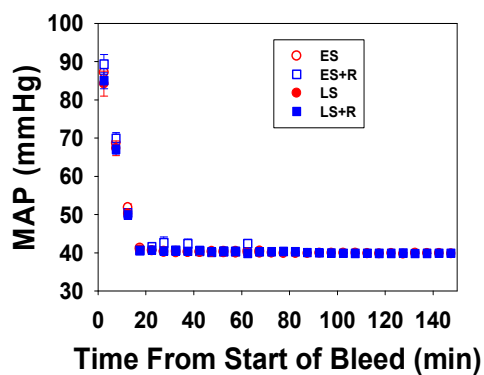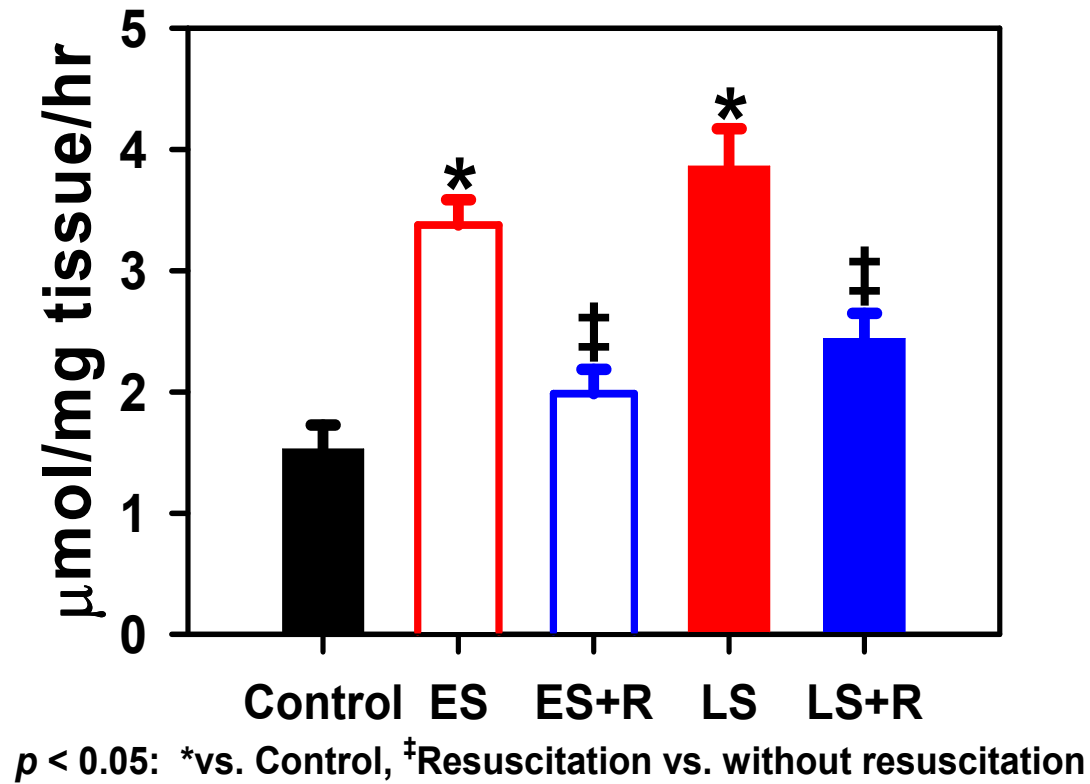

C: Mean Arterial Pressure during Resuscitation
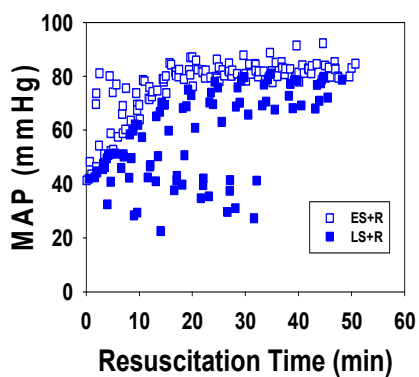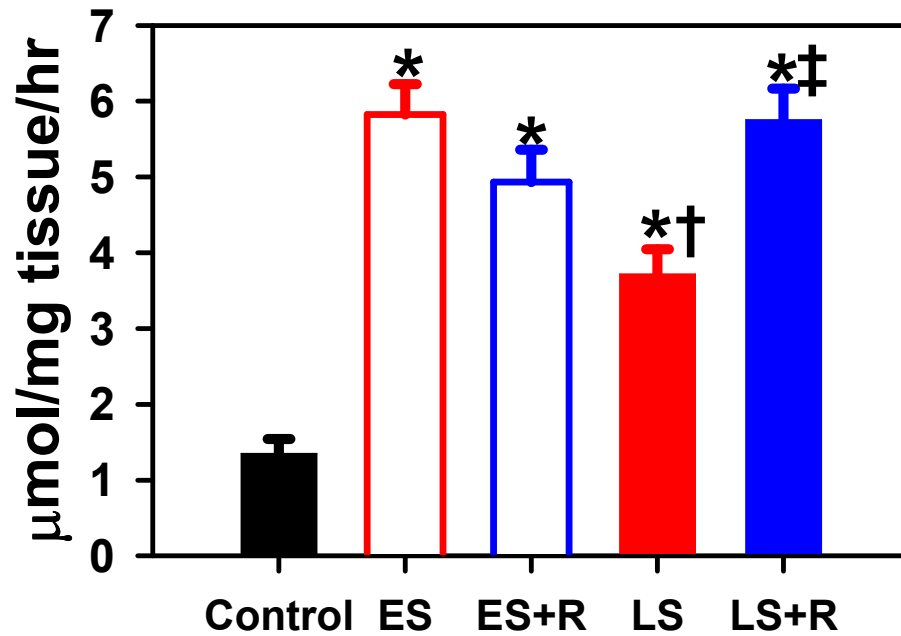

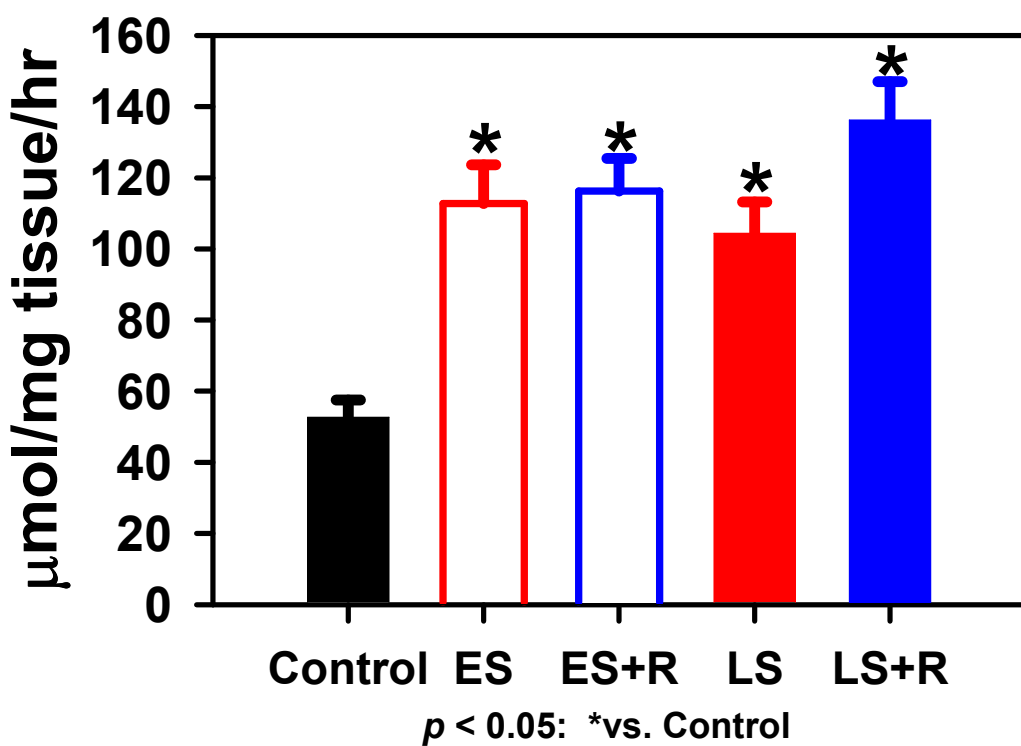**D: Resuscitation Volume vs. Time**

Fig. 2: Skeletal Muscle NKA Activity

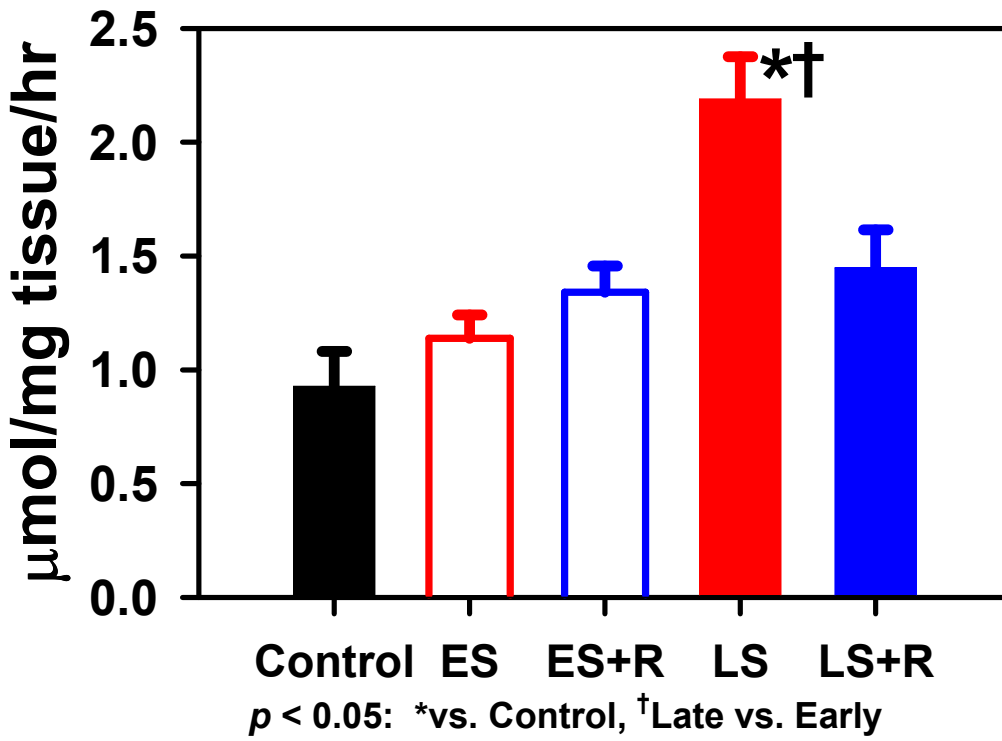$p < 0.05$: *vs. Control, ‡Resuscitation vs. without resuscitation
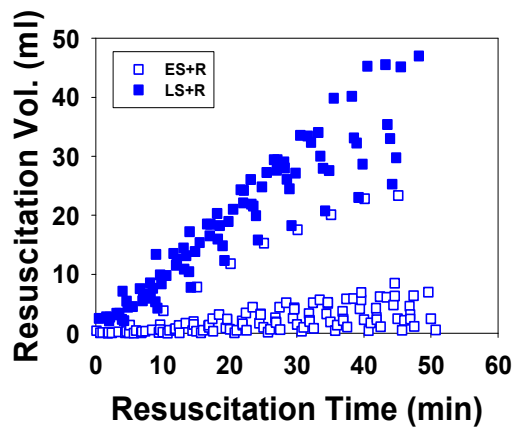
Fig. 3: Left Ventricle NKA Activity

p < 0.05: *vs. Control, †Late vs. Early, ‡Resuscitation vs. without resuscitation

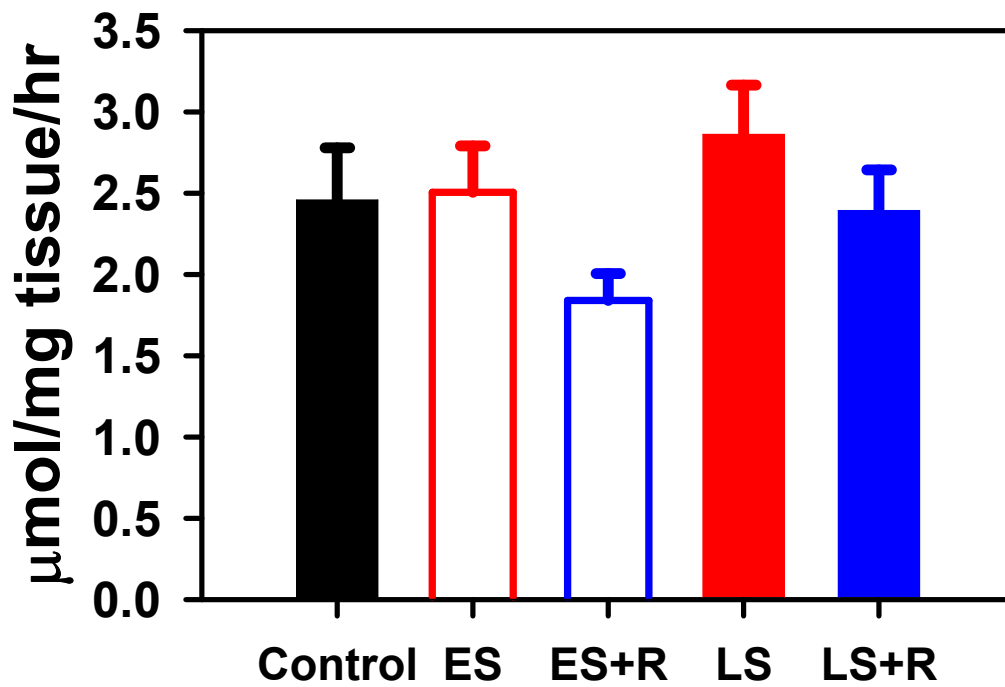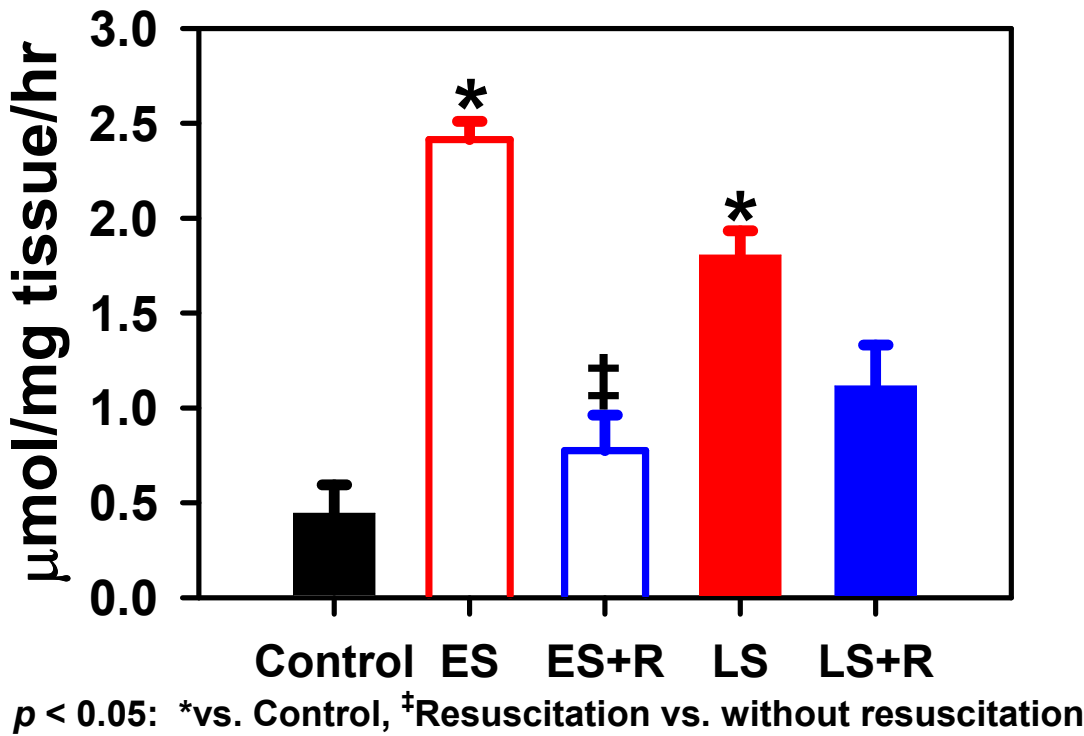**Fig. 4: Kidney NKA Activity**

*p < 0.05: *vs. Control

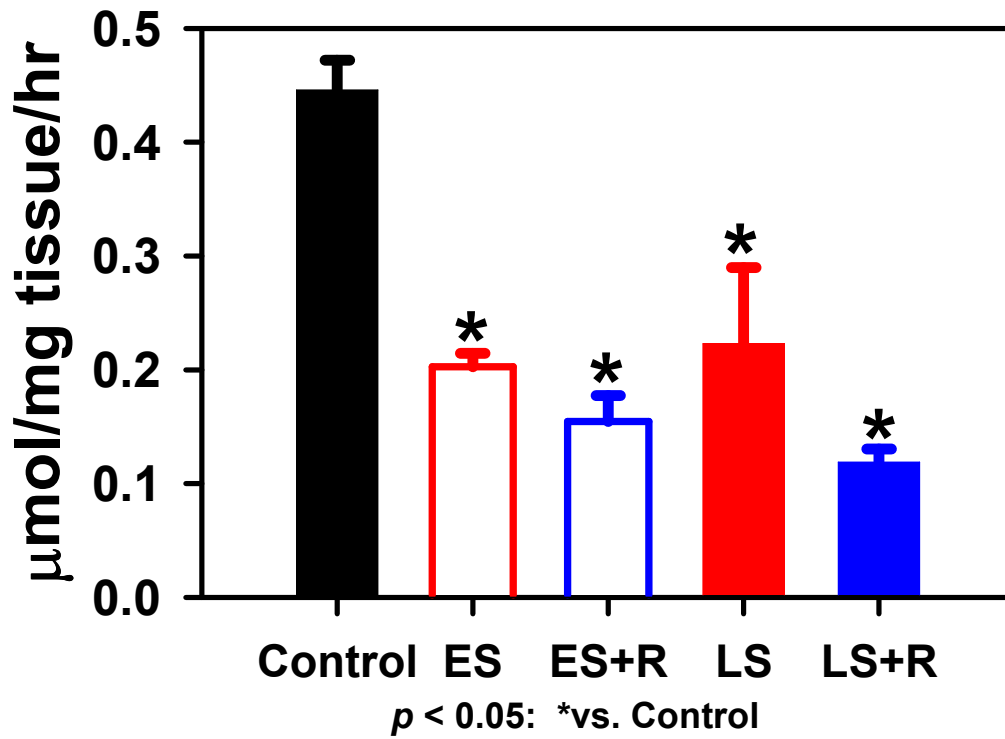# Fig. 5: Diaphragm NKA Activity

μmol/mg tissue/hr

2.5
2.0
1.5
1.0
0.5
0.0

Control   ES   ES+R   LS   LS+R

*†

$p < 0.05$: *vs. Control, †Late vs. Early

Fig. 6: Liver NKA Activity

Fig. 7: RBC NKA Activity

$p < 0.05$: *vs. Control, ‡Resuscitation vs. without resuscitation

Fig. 8: Plasma NKA Inhibitor

$p < 0.05$: *vs. Control

		Baseline	Start of Resuscitation	End of Resuscitation
ES+R (*n*=8)	MAP	96±8	54±4†	83±3†
	$(dP/dt)_{max}, (10^5)$	16.6±1.7	9.3±2.1†	15.0±2.1‡
	NKA	1.4±0.2	5.8±0.4†	5.6±0.6†
	NKA-I	0.39±0.05	0.20±0.01†	0.13±0.01†
LS+R (*n*=8)	MAP	110±4	42±1*†	64±8*†
	$(dP/dt)_{max}, (10^5)$	14.9±1.7	9.3±1.5†	14.8±1.9‡
	NKA	1.4±0.2	3.7±0.3*†	6.4±0.5†‡
	NKA-I	0.39±0.05	0.13±0.3*†	0.08±0.01*†

Table 3. Cardiac contractility measurements. $p < 0.05$: *LS vs. ES, †vs. Baseline, ‡End. Vs. Start of Resuscitation